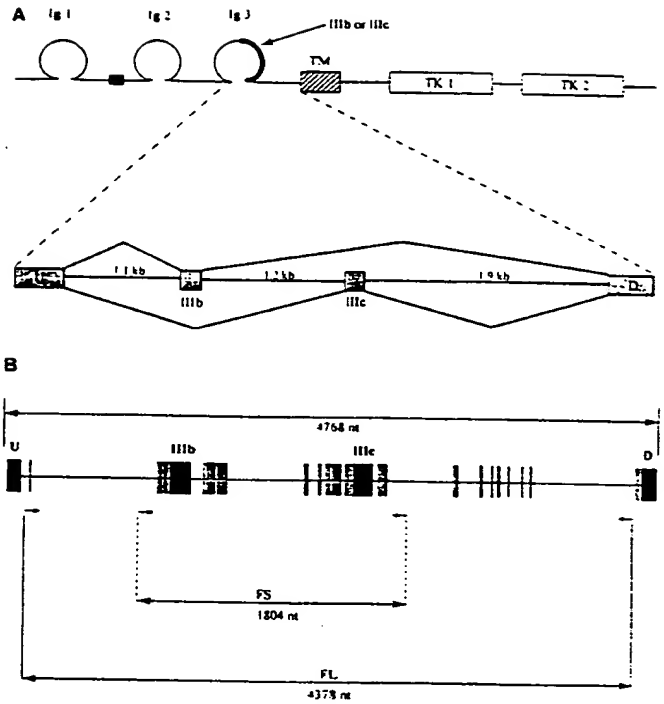


FIG. 1. Structural organization of the FGF-R2 gene and demonstration of IIIb and IIIc mutually exclusive splicing. (A) Organization of the FGF-R2 protein domains (top) and genomic gene arrangement of the region in which alternative splicing yields transcripts containing either the IIIb or IIIc exon and encoding the second half of the third immunoglobulin (Ig)-like domain. TM, transmembrane domain. TK, tyrosine kinase domains. The solid box represents a highly acidic domain, and the thick line indicates the IIIb- or IIIc-encoded portion of the protein. Shaded boxes represent exons, and solid lines represent introns, with intron sizes indicated. U and D indicate the exons upstream and downstream of these alternative exons, respectively. (B) Scale representation of the exons (solid boxes) and introns (solid lines) with regions of high (at least 90%) rat-human intron sequence similarity (shaded boxes). Also shown are regions FS and FL and their sizes. nt, nucleotide.

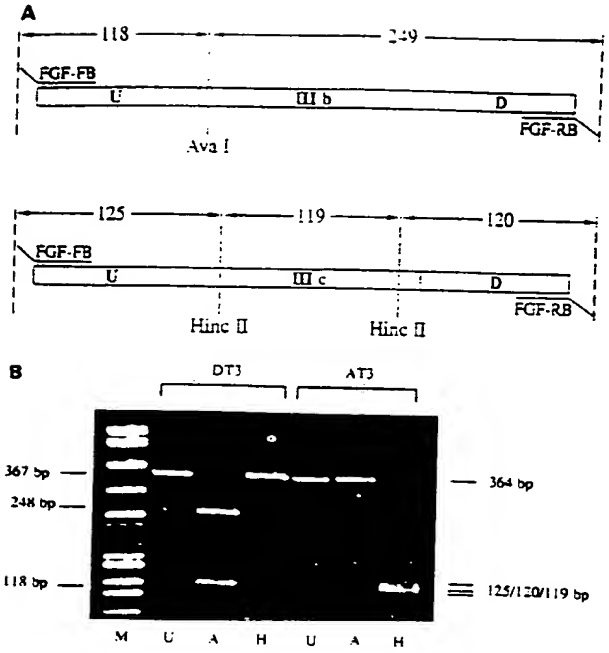
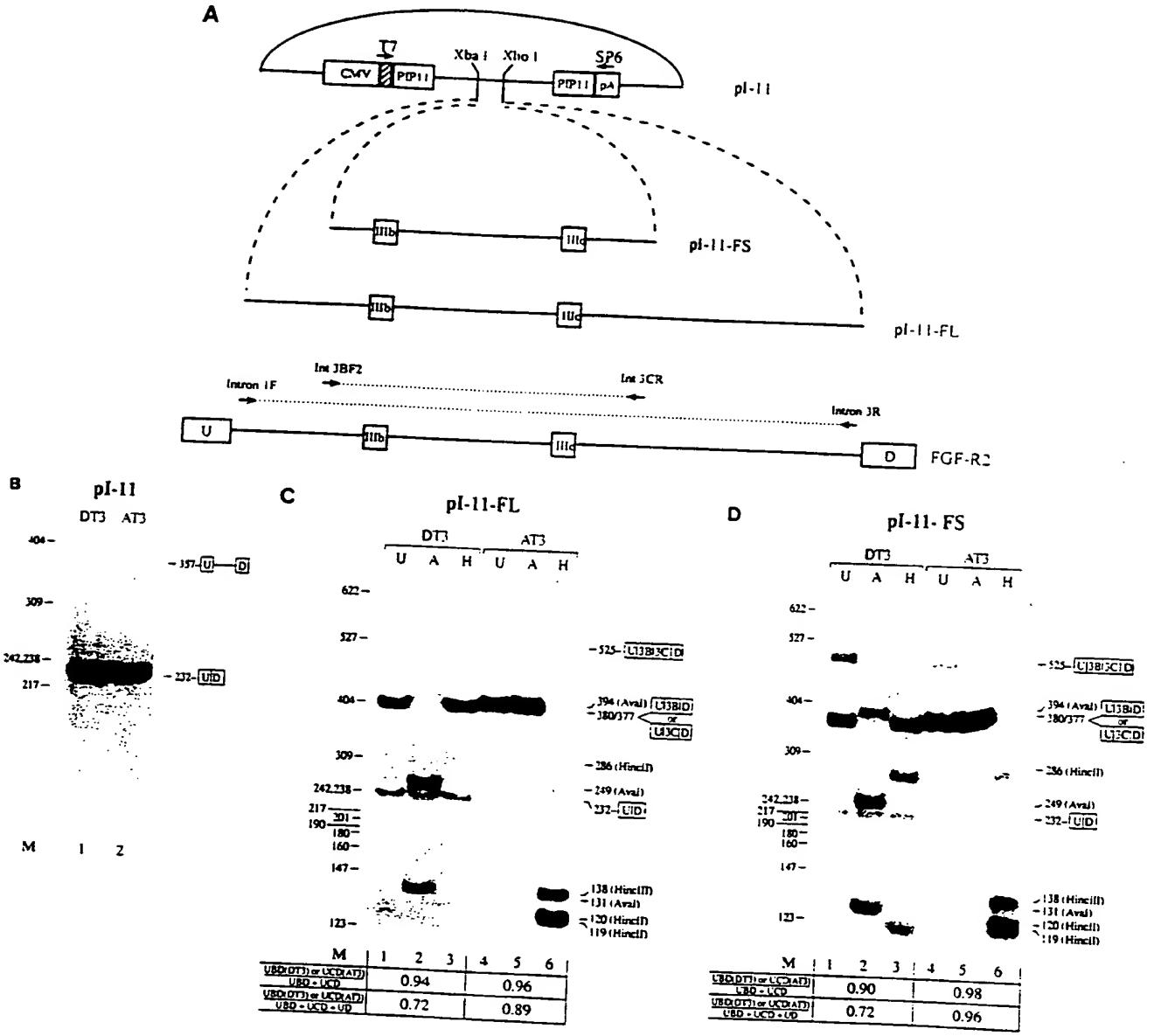


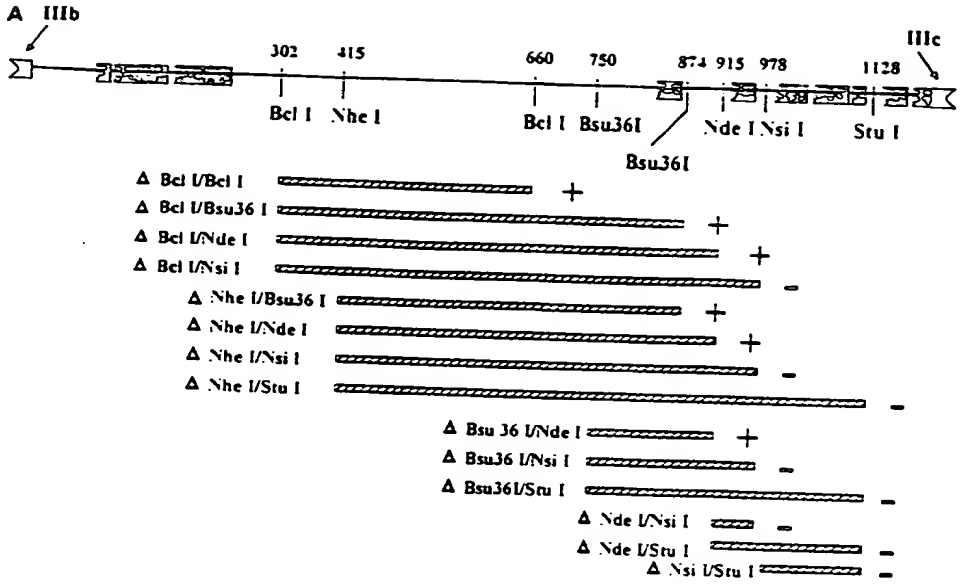
FIG. 2. Splicing of the endogenous gene transcript in DT3 and AT3 cells. (A) Map illustrating PCR products containing exon IIIb or IIIc amplified with primers FGF-FB and FGF-RB and sizes (in nucleotides) of fragments which result from *Ava*I or *Hinc*II digestion. U, upstream exon; D, downstream exon. (B) Gel showing the RT-PCR products following digestion with *Ava*I and *Hinc*II. DT3 cells express only products containing IIIb, and AT3 cells express products containing IIIc. U, uncut products; A, *Ava*I-digested products; H, *Hinc*II-digested products; M, pBR322 Msp I DNA size markers.

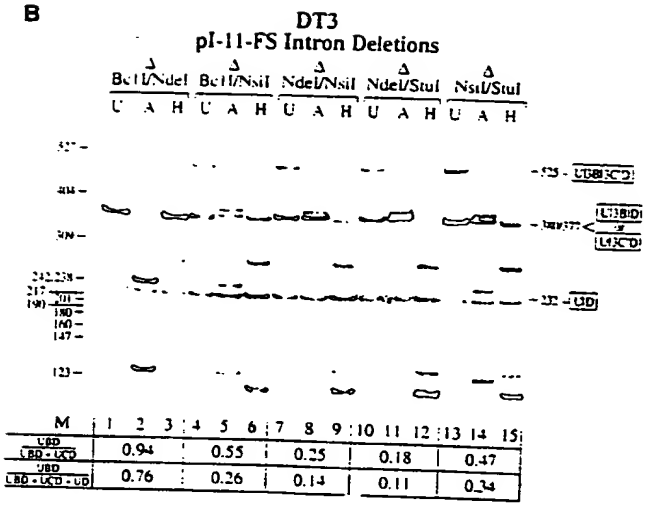


E

	Uncut	Ava I	Hinc II
U B C D	525	394 131	286 120 119
U B D	380	249 131	380
U C D	377	377	138 120 119
U D	232	232	232

FIG. 3. Rat FGF-R2 minigenes transfected into DT3 and AT3 cells reproduce the splicing pattern of the endogenous gene. (A) Representation of the two-exon, one-intron splicing construct pI-11 and insertion of FGF-R2 genomic sequences FL and FS (which were generated with the primer sets indicated at bottom) to create minigenes pI-11-FL and pI-11-FS, respectively. CMV indicates the efficient immediate early CMV promoter, and pA indicates the bovine growth hormone polyadenylation sequence. The *Xba*I and *Xho*I sites used for cloning and the T7 and SP6 vector-specific primers are also indicated. U, the 5' exon of pI-11; D, the 3' exon of pI-11. (B) pI-11 pre-mRNA is spliced almost completely and with equal efficiency in DT3 and AT3 cells, indicating no differences in the abilities of these cells to splice the exons. RT-PCR products for this and subsequent minigenes were obtained with the T7 and SP6 promoter primers. (C and D) Minigenes pI-11-FL and pI-11-FS reproduce the endogenous gene splicing pattern. The major PCR product containing either IIIb (3B or B) or IIIc (3C or C) is 380 or 377 bp, respectively. Products containing exons U, IIIb, IIIc, and D are indicated to the right. The sizes of products of *Ava*I and *Hinc*II digestion are also indicated. Quantification was performed to yield values for the fraction of the expected IIIb (in DT3) or IIIc (in AT3) exon as a fraction of products containing IIIb and IIIc and also as a fraction of products skipping IIIb and IIIc (see Results and Materials and Methods). (E) Representation of the origins (in nucleotides) of the products obtained when UBD, UCD, and UBCD products are cut with *Ava*I and *Hinc*II. Sizes are indicated in base pairs. Lanes are labeled as in Fig. 2.





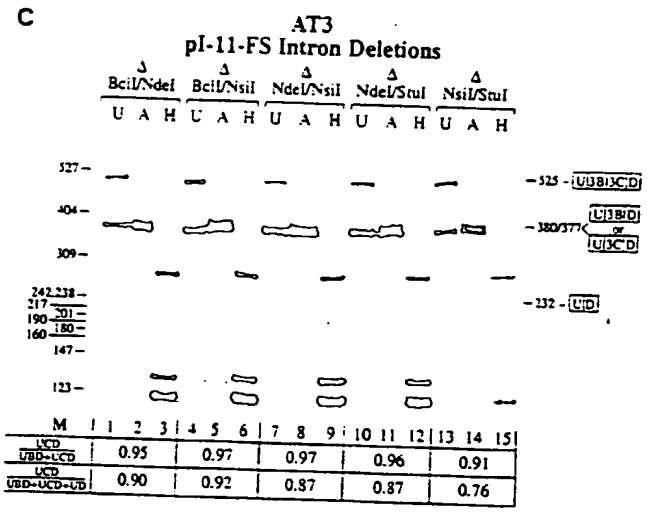


FIG. 4. Deletions which result in loss of sequences between the *NdeI* and *NsiI* sites in intron 2 result in loss of regulation in DT3 cells. (A) The IIIb and IIIc exons (solid boxes) and the intron (intron 2) between them (solid line) are shown. Also indicated are the restriction enzymes used to generate these deletions and the regions of high rat-human sequence homology (shaded boxes). The locations of these restriction sites are represented as the position (in nucleotides) from the start of the intron and are measured to the center position of each recognition sequence. The minigenes tested consisted of deletions (hatched boxes) from the parent construct, pI-11-FS. and the results of these deletions in DT3 cells are summarized. Delta. construct in which the sequence between the indicated restriction enzymes was deleted from pI-11-FS: plus. deletion constructs which still demonstrated >80% IIIb inclusion in DT3 cells: minus. deletion constructs with ≤55% IIIb inclusion in DT3 cells. (B) Results of the most representative intron 2 deletions in DT3 cells. Deletion of over half of the intron from *BclI* to *NdeI* did not affect regulation. whereas deletions spanning *NdeI* to *NsiI* caused loss of regulation. A deletion of *NsiI*-to-*StuI* sequences also caused some loss of regulation. but less than a *NdeI*-to-*NsiI* deletion. (C) The same deletions had no effect upon splicing in AT3 cells. Efficient IIIc usage was seen in these deletions. as well as all deletions summarized in panel A. Abbreviations are defined in the legends to Fig. 2 and 3.

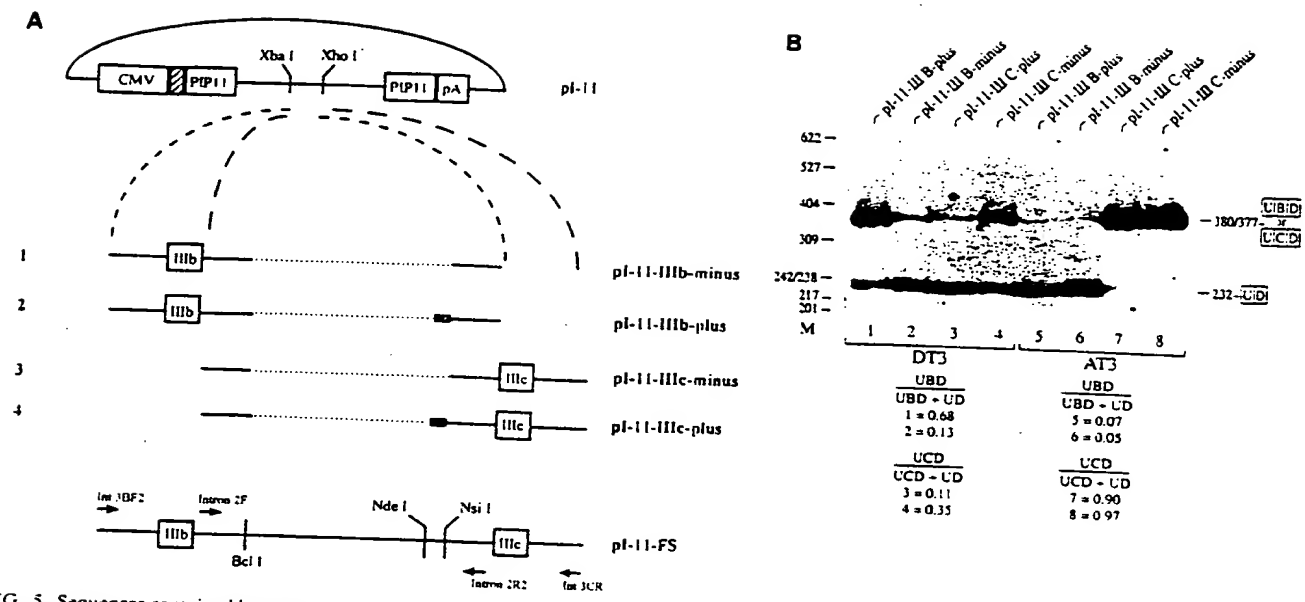


FIG. 5. Sequences contained between the *Nde*I and *Nsi*I sites of intron 2 normally function to activate upstream IIIb splicing and repress downstream IIIc splicing. (A) Method used to generate minigene constructs containing either the IIIb or IIIc exon with *Nde*I-to-*Nsi*I sequences (crosshatched boxes) present or deleted. All constructs had sequences *Bcl*I to *Nde*I, which were previously shown to be dispensable for regulation, deleted. The primers used to generate these regions in relation to the sequences of pI-11-FS are shown. The hatched box represents polylinker sequences present in PCDNA 3. (B) Transfection of the minigenes into DT3 and AT3 cells reveals that AT3 cells use exon IIIc highly efficiently and do not use exon IIIb efficiently regardless of the presence of *Nde*I-to-*Nsi*I sequences. DT3 cells use exon IIIb efficiently only when these *Nde*I-to-*Nsi*I sequences are present downstream. DT3 cells do not use exon IIIc efficiently, but when these sequences are deleted, IIIc usage triples. Quantifications were performed as described in Materials and Methods. Abbreviations are defined in the legend to Fig. 3.

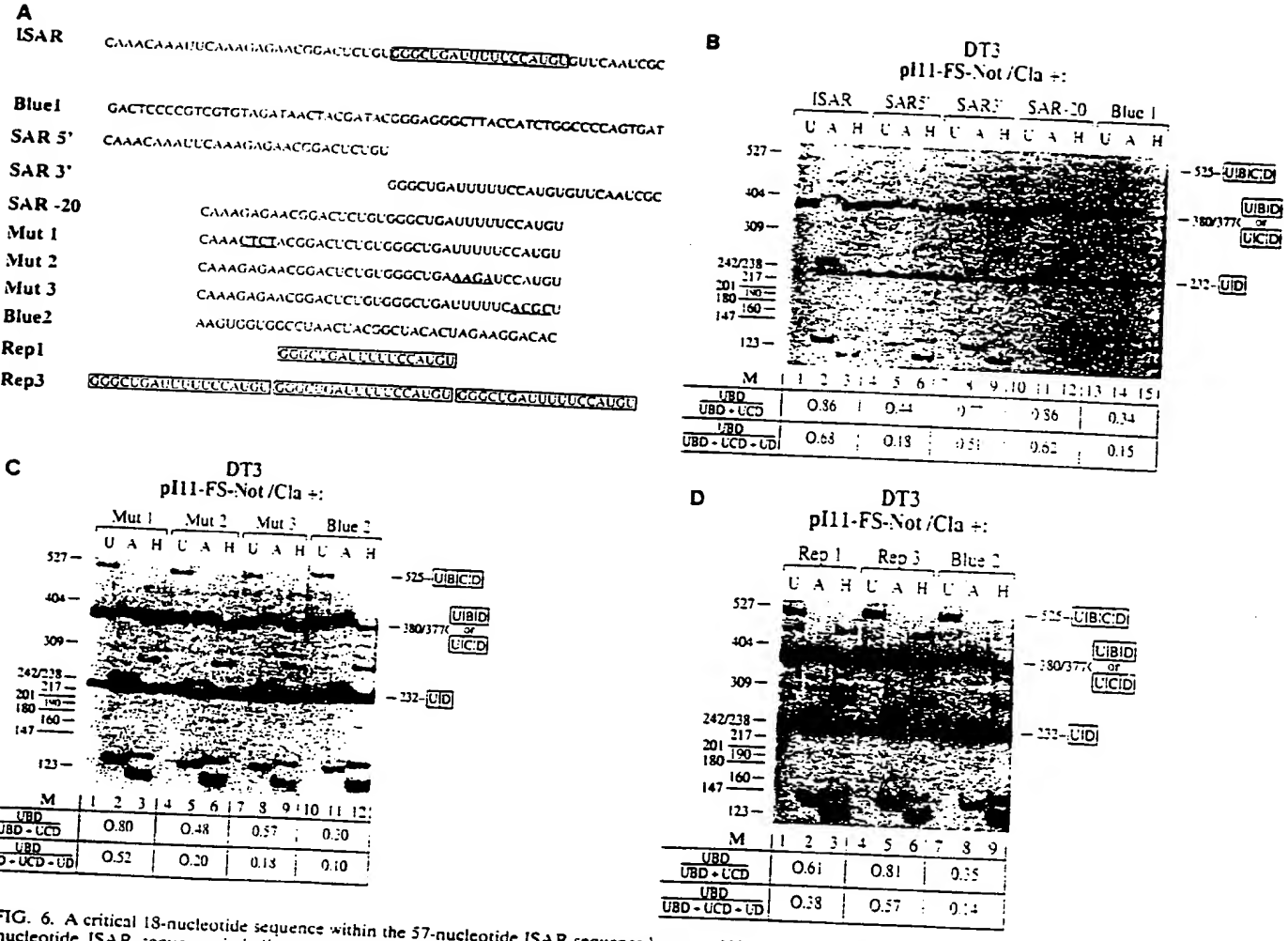


FIG. 6. A critical 18-nucleotide sequence within the 57-nucleotide ISAR sequence between *Nde*I and *Nsi*I nearly restores splicing regulation in DT3 cells. (A) The 57-nucleotide ISAR sequence is indicated at the top, and deletions and mutants of this sequence are shown below, as are control pBluescript sequences. The 18-nucleotide core sequence (Rep1) is boxed, and mutant sequences are underlined and in boldface. All sequences were tested by deleting ISAR sequences from pI-11-FS/Not/Cla-ISAR and inserting the indicated sequences. (B) SAR-20 and SAR 3' sequences restore regulation, whereas SAR 5' does not. (C) Mutations in the 18-nucleotide sequence shared by SAR-20 and SAR 3' (Mut2 and Mut3) cause loss of regulation, whereas a mutation outside this region (Mut1) preserves regulation. (D) One or three copies of the 18-nucleotide core sequence restore splicing regulation, with three repeats of the sequence being slightly more efficient than one repeat. Abbreviations are defined in the legends to Fig. 2 and 3.

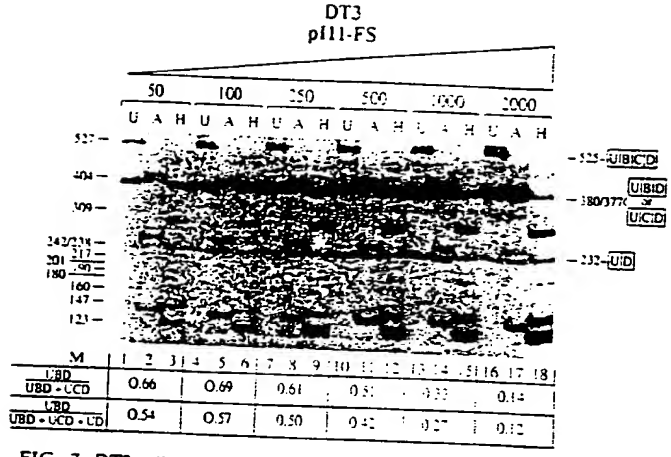


FIG. 7. DT3 cells contain a titratable factor or factors required for appropriate splicing regulation which can be overcome in transient transfections. Transient transfection of DT3 cells with increasing numbers of pI-11-FS minigenes resulted in stepwise loss of IIIb inclusion and increased IIIc inclusion, suggesting that a factor or factors required for regulation (i.e., IIIb inclusion and/or IIIc exclusion) is overwhelmed when large numbers of these minigenes are transfected. Abbreviations are defined in the legends to Fig. 2 and 3.

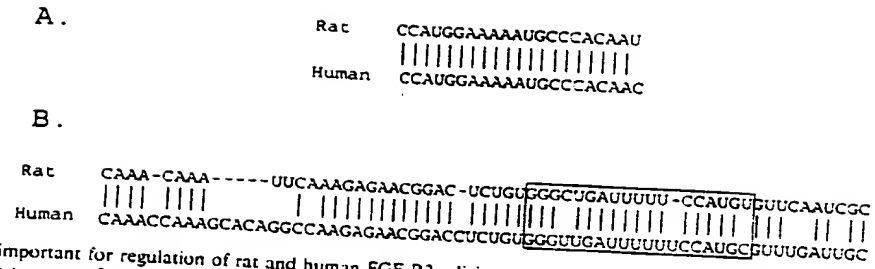


FIG. 8. Intron sequences important for regulation of rat and human FGF-R2 splicing are highly similar. (A) Rat intron sequences corresponding to a previously reported 21-nucleotide human sequence, IAS2 (see Results), which also mediates IIIb activation, contain only 1 nucleotide difference. (B) The 57-nucleotide rat ISAR sequence is highly similar to human sequences in this same region, including the 18 nucleotides shown to be most important for regulation (boxed sequences).

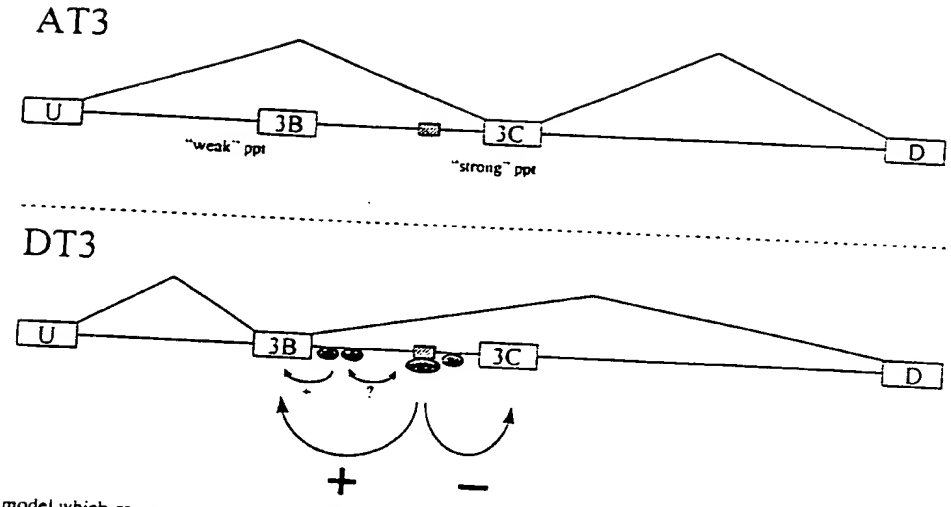


FIG. 9. Depiction of a model which can account for our results and the high fidelity of FGF-R2 splicing. AT3 cells use a default splicing pathway and choose the IIIc exon because of its stronger polypyrimidine tract (ppt); they splice IIIb inefficiently due to its weaker polypyrimidine tract. DT3 cells require a regulatory factor(s) which can activate (+) the weaker IIIb exon and at the same time repress (−) use of the IIIc exon. The ISAR element (indicated by a hatched box) is shown binding a factor or complex of factors (large shaded oval) which mediates both of these effects. The previously demonstrated contributions of other *cis* elements and associated factors (smaller shaded ovals) to IIIb activation are also shown, as well as the suggestion of possible cooperative interaction between proteins bound at several locations within the intron. Abbreviations are defined in the legend to Fig. 3.

GTAAC AACGTTTTTG TGTTTGTGTT

TTTTATTTTT TATTTTTATT TTTTTTTTTA AGAAAACTGA ATATAGGAGT

TAAAAAAGAC TCGGTGCTTT GGGAGGCAGC AGGCAGCTTC TAGAATAACT

CTTGTGGTCT TGGTATATTT ATAATGATCT TTCTTTGGTG GTGCAGCTGG

CGTCATGCCA GTGGCCATGG AAAAATGCCC ACAATGTTCA AAGTGCTTGA

AGATTATCTT CCACCCCCAC CCTGTTTTCA AGCCCTTCTT TCTGGTCTGT

CTTGTTTGGA CTGCACACTT CCCGTGATCA CTGTGTCTGA GTGCACGTGG

GCCTTGCGTT TGCATGCCCG TCGAGTCTGC ACTCTCTGAT TATTAAGCCA

GACTTGGTTG CCTTTTATGC TAGTGACATA GAGAAATGCT AGCATGATAG

GATTCACCTA ACGAAAGTTT TGTTCTTTGG TTCGATTCCA CACCGGATCC

TTTCCAAAAC TGGAGAATGG TTATCTTCTA GTGCGTATGA CACTGGAGGA

TAGTGAAGGC AGATGGTGGA GTTTTCAGTT ATCATTCTTC ACACGCAGAC

ATATTCATAT TAGAAAAGGA AACAAACCAT AAATCCAGTT TTTTCTGTTA

CCAGTATTAC ACTTTCTGCC ATGTTCTTTC AATGATCATA TAAAGCAAGA

TGATTTTCGG CCTGAATGAA ATTAACCAGA ATCCAGTCAC CAAGATAAAG

TCCCACCCTG GTTCCCATGG AGCCTGAGGG ATGTGTGGGA TGTCCACCTG

ATCTGCCGTG CTTTATTCCA TCACACAGAA AATAGAAGAG CCTCCCCTTT

TCTCACAATT GGAGTCTGCA TCCAACAGGA CCAGAACCCA GATTAGCCCT

CAGGGTATTA TACTTTTTGG AAACCCACTC CCAAATCCAT ATGCAAACAA

ATTCAAAGAG AACGGACTCT GTGGGCTGAT TTTTCCATGT GTTCAATCGC

ATGCATGTCT AAGGTGGTGA CGCCGGTGTG GTGATGGGCC TGCAGAGGTG

AGCTGGCCGG TGTCTCTCAG TGTCTCTTGG TTGTGGGCTT TGTGGACGGG

CTGCAGTTGG AATCTCCTGA TGGCCAGCAC CCCCTGGACC TGCTGGGACA

AGGCCTCTTG GTTCCAAGGC CCCCTCCACA ATCATTCCTA TGTCTAGCCT

TTTTCTTGCT TCGTTTGTTT TCTAG

Fig. 10

1 →GTAACAAT GCTTCATTTT TGTCTTTTTT TAAAAAGAAA GCTGGATATA

GAAGCTGAAA AGACTTGGTG CTTTGGGAGA CTGCAGGCAG CTTATAGGAT

AACTCTTGTG GCCTTGGTAT ATTTATAATA ATCTTTCTTC GGTGATGCAG

CTGGTATGAT GCCAGTAGCC ATGGAAAAAT GCCCACAACG TTCAAAGTGC

TTGCTCCAAT TTCTTCTAGA GATTAGCCTC CACCCCCACC CAGTTTTTAA

GTTGTTCCTT CTGGTTGATC TTGTTTAGGC TGCACATTTC CCATCATTAC

TGCACATTAA CACCATTTAA AACACACGCT TCCATGCCTG TTTAATACGG

GGCATTTGAA TATCAGCAGA GTTTGTCCAA GTTTTTAGGG AAATATTGGC

AAGATGCAAT TTGTTCAACA AAGCATCATT TCTTTGGTTG CATGGTTGAT

CCTTATGAGT TGCTGTTCTT GACCTTGTTG CACCAAATTT GAGGGGAGCT

CATCTTAATG AATGTACTAC TGGACGCTAC TAAAGGCAAA AGGTTGACTT

TTTAGGTTTG TCATGACTCA CATCCAAATG TTTATTAATG AAAAGAGAAA

AAGCCCAGTT TTTTTGGTTA CCAAGATGAT GCTTGCTTCC ATTTCTTTTT

GTCAATGCTA TGTAGGGCAA GATGGTATCG CAGAAGTAAA AATAACCAGA

GCCTGGTAAC CAAGACAACC TTCCACCCCA ATTGGTTCCC ACAGGGCCAG

GAGGATGGGT GAGGTGCCCA TCTGGGCTTA TGTGCAGTGT GTTGTCTTAA

AACACAGCAA TTTAGATAGA ACTACCCTTT CCTCTTGGTG GGAGTCTGCA

GCCAACAGGA CCAGAACCAG CTTGGCCTTC TGGGCACCAT ACTTTTGGAA

AACCACCCCT AAATGCAAAC CAAAGCACAG GCCAAGAGAA CGGACCTCTG

TGGGTTGATT TTTTCCATGC GTTTGATTGC GTGCATGTGT AGGAGGTGAA

GCCGGTGTGG TGACGGGCCT GTGGAGGTGA GCTGGTCAGT GTTGCTCCGT

GTCTCTCGGT TGTGGGACTT TGTGGATGGG CTGCAGTCGG AATCTCCCAG

TGGCCAGCAC CCCCTGAAGC CCCCGGTGCG ACGCCTTGTG GTTCCACAGC

CCCCTCCACA ATCATTCCTG TGTCGTCTAG CCTTTTCTTT TGCTTCCCTT

GTTTTCTAG

FGFR2 Gene: exons

Human intron between IIIb + IIIc

Fig. 11